

# Improved Oral Adsorption of CPC Shows Improved Anti-Microbial Activity

John Coggan\*, Deepa Khambe, and Roger Gibb

P&G, Mason, OH, USA

0569

## ABSTRACT

**OBJECTIVE:** To evaluate whether differences in oral adsorption of CPC correlate with antimicrobial activity. **METHOD:** 10 subjects participated in a randomized crossover mouthrinse dosing study. Each subject dosed with 5 mL of 4 different CPC-containing mouthrinses on different days. Oral lavages were collected before and 90 minutes after treatment. Alamar Blue™, a dye that produces a fluorescent compound when metabolized by bacteria, was used to measure microbial activity. Mouthrinses exhibiting antimicrobial activity have lower fluorescence for lavages collected after rinsing relative to those collected before rinsing. Adsorption of CPC in the oral cavity was quantified via HPLC from mouthrinse expectorates. **RESULTS:** Amount of CPC adsorbed in the oral cavity and decreased microbial activity correlate well.

## INTRODUCTION

Many mouthrinse formulations contain the active ingredient cetylpyridinium chloride (CPC) to reduce plaque and gingivitis. CPC is thought to adsorb to the cell walls of bacteria and kill the bacteria by cell lysis. It has been proposed that increased levels of CPC adsorption to the oral mucosa and salivary debris correlate with increased antimicrobial activity and efficacy.

We have developed an in vivo method that measures the adsorption of CPC in the oral cavity after rinsing with a mouthrinse. We measure the non-adsorbed CPC in mouthrinse expectorates by HPLC with UV detection. Antimicrobial activity is determined by adding a dye that fluoresces in the presence of metabolically active bacteria present in oral lavages collected before and after rinse treatment. If a mouthrinse has antimicrobial activity the oral lavages collected after treatment should fluoresce much less than those collected before treatment. The difference in fluorescence is used to calculate antimicrobial activity.

## PURPOSE

To demonstrate that increased oral adsorption of CPC correlates with increased antimicrobial activity after administration of four (4) different CPC-containing mouthrinses.

## MATERIALS AND METHODS

### Study Design

Ten (10) subjects participated in a randomized crossover mouthrinse dosing study. Each subject rinsed with 5 ml of each CPC mouthrinse on different days. Subjects refrained from mouthrinse or any other antimicrobial treatment for a 24 hour washout period.

### Equipment and Materials

Cetylpyridinium Chloride and Glycerin -USP grade

Alamar Blue™ - Biosource

HPLC- Waters 2695 with a 2487 UV detector and Sedex evaporative light scattering detector set in series.

Fluorometer- Spectramax Gemini

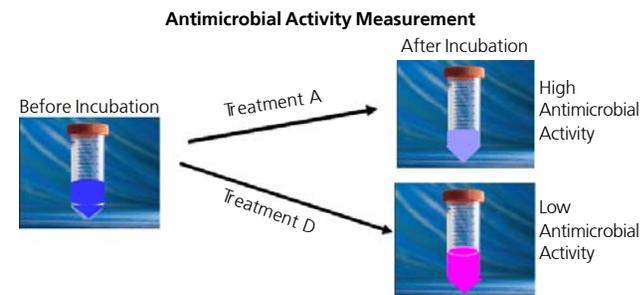
### Oral Adsorption Measurement-

1. After rinsing with mouthrinse, subjects expectorated into collection tubes.
2. Samples were centrifuged @ 5000 rpm for 5 minutes
3. Aliquots of the supernatants and dosing solutions were analyzed for CPC by HPLC with UV detection at 265 nm using glycerin as an internal standard.
4. Amount of CPC adsorbed to salivary debris and the oral mucosa was determined by the difference between the amount of CPC in the supernatant (non-adsorbed CPC) and the total amount of CPC dosed.

### Antimicrobial Activity Measurement-

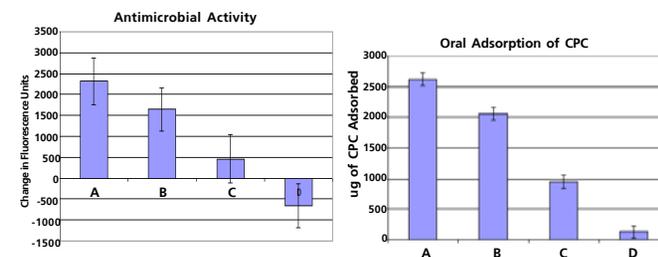
1. Oral lavages (10 ml water) were collected before and 90 minutes after rinse treatment.
2. Serial dilutions up to 1:10 of the lavages were made with isotonic phosphate buffered solution (PBS).
3. Aliquots of a 1% Alamar blue were added to each solution and incubated at 37° C for 3 hours in a water bath.
4. Aliquots of the incubated solutions were placed on 96 well microplates in triplicate and the fluorescence measured. (Excitation = 485 nm, Emission = 590 nm)
5. Antimicrobial activity was determined by measuring the difference in fluorescence of lavages collected before (highly fluorescent) and after rinsing (low fluorescence).

## MATERIALS AND METHODS (Cont.)



The mean level of CPC adsorbed to solid surfaces in the mouth and the difference in fluorescence were modeled using separate general linear models. There was no evidence of carryover in either model. Results are shown below.

Formulation	A	B	C	D
Mean Change in Fluorescence	2318 ± 586	1643 ± 586	463 ± 586	-657 ± 522
Average µg CPC Absorbed	2617 ± 108	2059 ± 102	960 ± 108	127 ± 102



## CONCLUSION

Increased anti-microbial activity correlates well with increased oral adsorption of CPC.